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16. ABSTRACT

The objective of the project was to evaluate control measurements that might be made at land treatment sites to determine the effectiveness of operation in the management of hazardous wastes. Initial studies were on measurement techniques available for parameters to serve as indicators of biodegradation for a land treatment facility. An earlier paper reported results of comparative studies between wax impregnated graphite electrodes (WIGE) and platinum wire electrodes (PWE) to determine ODR and redox potential (Eh). The study indicates that WIGEs are less susceptible to oxide poisoning, easily fabricated, relatively inexpensive, and more stable in soil than PWEs. This paper reports results of using WIGEs and PWEs to measure Eh and ODR during biodegradation of glucose and phenol in a soil environment as compared to determining actual biodegradation of those compounds by analytical procedures. The results indicate that Eh and ODR can be suitable parameters for indication of biodegradation of organics by land treatment processes.

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REDOX POTENTIAL AND OXYGEN DIFFUSION RATE AS PARAMETERS FOR MONITORING BIODEGRADATION OF SOME ORGANIC WASTES IN SOIL

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ABSTRACT. Wax impregnated graphite electrodes (WIGEs) and platinum wire electrodes (PWEs) have been used to monitor the redox potential (E_H) and the oxygen diffusion rate (ODR) during biodegradation of glucose and phenol in a soil environment. The microbial activity and the actual degradation of these compounds also were determined analytically, and the results were compared with the E_H and ODR measurements. The results show that E_H and ODR can be suitable parameters for the indication of complete biodegradation of organic wastes by land treatment.

INTRODUCTION

Degradation of organic wastes in soil is a complex process. The rate and the extent of biodegradation depends upon physiochemical factors such as temperature, moisture content, oxygen supply, pH, carbon-to-nitrogen ratio, particle size and the chemical constituents of the soil, the loading rate of the wastes, the type and the concentration of microorganisms in soil, and other factors (1).

During the past two decades, a significant amount of research has been conducted to understand the mechanisms of biodegradation of a number of pesticides and some industrial wastes (2). Some general patterns of biodegradation processes appear to have emerged (3,4) from these studies. However, it also is obvious that under natural environmental conditions, the interdependability of many of the abovementioned factors may alter the degradation pathways quite significantly. The aeration and water status of soil are two of the most significant soil parameters that can influence the biodegradation of hazardous wastes. An adequate supply of oxygen activates the aerobic microorganisms, resulting in rapid degradation of organic compounds to carbon dioxide and water; oxygen-depleted conditions are favorable to anaerobic microorganisms that may decompose the organic compounds by different mechanisms to smaller molecules such as methane, amines, etc. (5). Since in a landfarm both aerobic and anaerobic conditions can be created conveniently either by plowing the soil or by controlling the water content, it appears that time-dependent measurements of oxygen availability and the redox condition of the soil can be suitable indicators for the extent of biodegradation of hazardous wastes.

A review of the literature indicates that the mechanistic aspects of the biodegradation of hazardous wastes has been the focus of major research ac-

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tivities. The biodegradability of various compounds was investigated by controlling many of the above-mentioned factors, and the results were verified through periodic analysis of soil for various constituents of the added wastes. It has been postulated (1) that redox potential (E_H) may be an excellent indicator of the extent of biodegradation in soil. It also has been demonstrated that controlled E_H and pH have profound effects on the rate of biodegradation of some pesticides in soil suspension (6). In this paper, we present results of laboratory studies involving measurements of redox potential and oxygen diffusion rate (ODR) as "*in situ*" monitoring techniques for biodegradation of some organic wastes.

ODR and E_H measurements in soil environments are generally measured electrochemically using platinum microcathodes (7,8). It has been well documented (9,10) that platinum and gold electrodes are "poisoned" in aqueous and soil environments due to the formation of oxide and sulfide coatings, thereby becoming chemically active, which results in inaccurate measurements of the above parameters. This drawback of metallic electrodes led to the development of graphite-based electrodes, which were used extensively in aqueous and nonaqueous solutions (9,11–13). Recently, we have demonstrated (14) that wax-impregnated graphite electrodes (WIGEs) performed better than platinum wire electrodes (PWEs) for the measurement of ODR and E_H in soil environments. This paper examines the results of studies on the biodegradation of glucose and phenol as monitored by both WIGEs and PWEs. Both glucose and phenol biodegrade appreciably under either aerobic or nonaerobic conditions.

MATERIALS AND METHODS

The fabrication procedures for WIGE and PWE, the preparation of soil prior to biodegradation studies, and the electrochemical techniques for measurement of ODR and E_H are described elsewhere (14). The soil was Ap horizon of Crowley silt loam obtained from Stuttgart, AR. The experiments were conducted at a laboratory temperature of 22 ± 1 °C.

Biodegradation studies of soil samples were conducted under two controlled soil-water content conditions: (1) At a soil moisture-content of 25% (W/W) and at −0.3 bar water potential: Aqueous solutions of organic compounds of desired concentration, containing 1% (W/W) ammonium nitrate to maintain the carbon-to-nitrogen ratio for proper microbial growth, were mixed uniformly with soil (14). The matric potential of the prepared soil samples was −0.3 bar as determined by water retention (15). (2) In water-saturated soil samples, which were prepared in two ways: [a] Soil samples of 25% moisture content (W/W) were prepared as above, to which another 10% (W/W) of water was added from the top to achieve saturation; previous studies in our laboratory indicated complete saturation of the soil at 35% moisture content by weight. Samples prepared in this fashion were found to have 1–2 cm of solution ponded on the soil after about 24 h, and this layer disappeared in about 10 days due to either infiltration into the soil and/or evaporation. [b] Solutions of organic compounds were prepared as above in volumes equivalent to a moisture content of 35% (W/W) of soil; the solution then was poured from the top. This situation represents the conditions which may exist in a landfarm; however, it took 15–20 days for the solution layer either to infiltrate or evaporate. The rate of infiltration of the solution into the dry soil was slow. Since the soil surface was ponded with the solution for longer periods of time, the soil in the column was effectively cut off from its source of oxygen in the air. The slow infiltration rate would also contribute to the variability observed in water and oxygen contents. Therefore, differences in sample preparation would cause differences in soil aeration which in turn would affect E_H and ODR. These parameters are indicators of the environment for microbial degradation of the organic compounds.

ODR and E_H were measured in containers containing 2.0 kg air-dried soil amended with organic compounds as described above. The depth of the soil in the containers was about 10–12 cm. ODR was measured in samples at 0.3 bar water potential, whereas E_H was measured in water-saturated samples. For ODR measurements, two PWEs, two WIGEs, one silver-silver chloride reference electrode (SSCE), and one brass anode were inserted at a depth of about 5–7 cm. A potential of −0.7 V was applied to the microcathode (PWE or WIGE) with respect to the SSCE; a steady state was reached after about 4 min when the current due to reduction of oxygen was measured. Measurement of ODR on all four microcathodes was performed successively, and the procedure was repeated every 2 days. No potential was applied during this interval so as not to reduce oxygen forcibly. The system was kept open to the atmosphere, so that equilibration with aerial oxygen would occur as in a natural environment and biodegradation could proceed under aerobic conditions. E_H measurements were performed, also once in 2 days, using two PWEs, two WIGEs, and one SSCE assembled in the same manner as described earlier.

Microbial activity was measured using another series of soil samples, each containing 100 g air-dried soil, prepared in a similar manner. Microbial activity was estimated as the amount of carbon dioxide released as a function of time. A manifold system, capable of handling six samples simultaneously, was constructed. Air was used as a purging gas which was first freed of carbon dioxide by passing through a 0.5

M KOH solution. The gas was then passed through the sample container, and released carbon dioxide was then carried over to another trap containing KOH of known concentration. The latter KOH solution was replaced periodically (usually every 3 days) with new solution and analyzed for carbon dioxide by precipitating with barium hydroxide solution followed by titration with standard hydrochloric acid (16). The flow-rate of gas in the manifold was controlled to 4–6 bubbles/min using precision flowmeters.

The extent of biodegradation also was determined by extraction of the organic compounds remaining in the soil. Another series of samples, each containing 100 g of air-dried soil were prepared similarly. Phenol was determined every 3 days using U.S. EPA recommended procedures (17). The soil sample was mixed with 150 mL of dichloromethane and refluxed for four hours in a soxhlet apparatus; the extract was concentrated in a Kuderna-Danish column, dissolved in hexane, and analyzed by flame ionization gas chromatography. Glucose was determined by the anthrone method (18). The soil sample was extracted in sulfuric acid, mixed with the appropriate quantity of anthrone solution, and the absorbance of the resultant solution was measured at 625 nm. Control soil samples not amended with organic compounds, but containing 1% (W/W) ammonium nitrate, were determined also by these methods. The pH was measured for another series of samples, each containing 0.5 kg air-dried soil and prepared in a similar manner; 20-g samples were collected periodically from each container, 20 g of water were added, stirred well, and then allowed to settle for one hour, followed by dipping the pH electrode into the aqueous layer above the soil.

RESULTS AND DISCUSSION

In an earlier paper (14), we showed that WIGE is a better choice than PWE for the measurement of both ODR and E_H in soil environments. It also is well documented that the effect of electrode poisoning is less significant with WIGE than with PWE. The poisoning factor and the chemical inertness become extremely important due to larger concentrations of organic compounds in a hazardous waste landfill. The effectiveness of WIGE and PWE, therefore, was tested in our laboratory using both probes in the same soil samples.

The data presented in Fig. 1 show the cumulative carbon dioxide released from soil samples amended with glucose and phenol at 25% moisture content. All samples showed significant amounts of microbial activity, with the highest CO_2 released by the 5% glucose sample and the lowest CO_2 released by the control as expected. The rate of CO_2 release was rapid during the first 5 days and decreased slowly

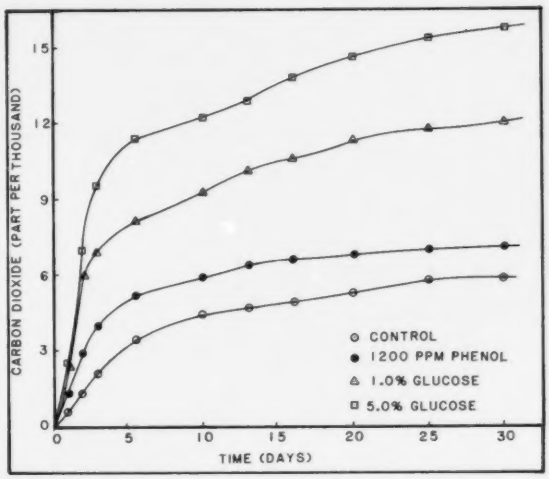

FIGURE 1. Microbial population in crawly siltloam soil amended with organic compounds at 25% (W/W) moisture content.

thereafter. Similar microbial activities were observed in soil samples containing 35% moisture content (data not shown), though at much slower initial rates, and the rates started leveling off after about 10 days. This observation was consistent with experiments involving extraction of glucose and phenol depicted in Fig. 2. Each datum in Fig. 2 represents the average of two extractions.

In water-saturated samples, the rates of biodegradation of glucose were significantly lower as compared to those at −0.3 bar matric potential, where no detectable amount of glucose remained after about ten days. However, there were no significant differences in degradation rates of phenol at the two moisture contents even though there were differences in microbial activity (Fig. 1). A possible explanation may be the initial toxic effects of the high concentrations of phenol on microorganisms as evidenced by relatively lower microbial activity of phenol samples

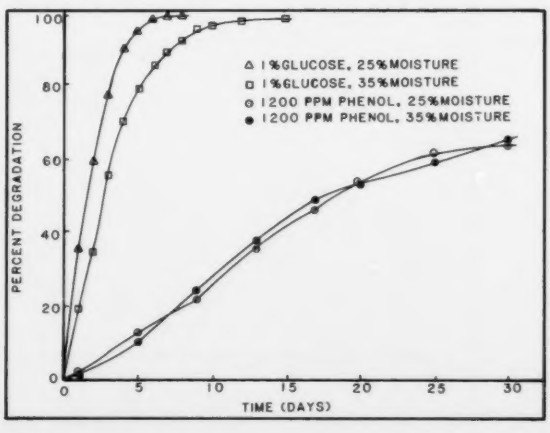

FIGURE 2. Degradation of organic compounds in crawly siltloam soil at different moisture contents.

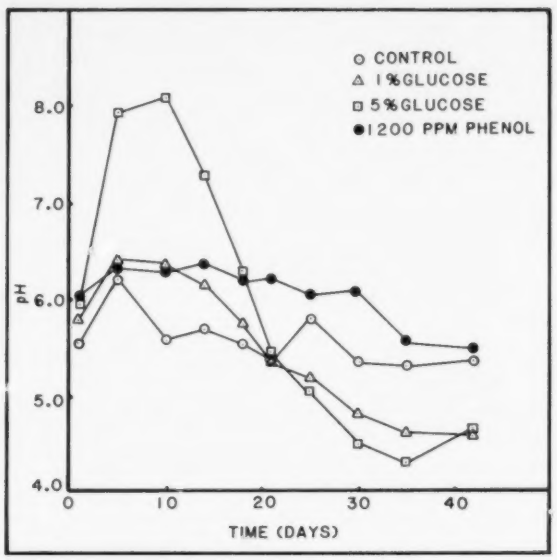

FIGURE 3. Variation of pH of soil samples amended with organic compounds during biodegradation.

as compared to that of glucose. Scott and co-workers (19) showed that high concentrations of phenol added to soil retarded the microbial activity. As phenol concentrations increased, the length of the lag phase increased logarithmically. While glucose samples are easily biodegraded by soilborne microorganisms, only about 60% of phenol samples are degraded after about a month.

The temporal variation of pH, E_H, and ODR during biodegradation are presented in Figs. 3–6, respectively. The variation of pH (Fig. 3) for glucose samples was significant, especially during the first 20 days. Although the reason for such a change is not clearly understood, it appears that rapid decomposition of glucose and its assimilation with chemical components of soil may have contributed to this phenomenon. This phenomenon is being investigated further in our laboratory. The observed E_H values are expressed with respect to those at pH 7, as recommended in the literature (20), for water-saturated samples prepared in two ways (see above). The data in Figs. 4 and 5 show that the measured E_H of the soil can be affected significantly by the method of sample preparation. In soil samples well amended with glucose followed by flooding, one notices that both PWE and WIGE recorded drastic changes in E_H. The initial decrease in E_H is attributed to the reducing environment due to the presence of organic compounds; as the degradation proceeded, a gradual increase in E_H occurred. Theoretically, complete degradation would be indicated when the E_H value reached that found in the control columns. In our studies, such a situation occurs for glucose, but only after about 30 days. Since complete biodegradation occurred in about 10 days (Fig. 2), this apparent disparity may be due to the slow response of the soil system with respect to redox equilibria.

The physical, chemical, and biological composition of soil is so complex that there may have been significant kinetic factors involving interchange between dominant redox components (e.g., Fe^{2+}/Fe^{3+}) which are responsible for changes in the redox poten-

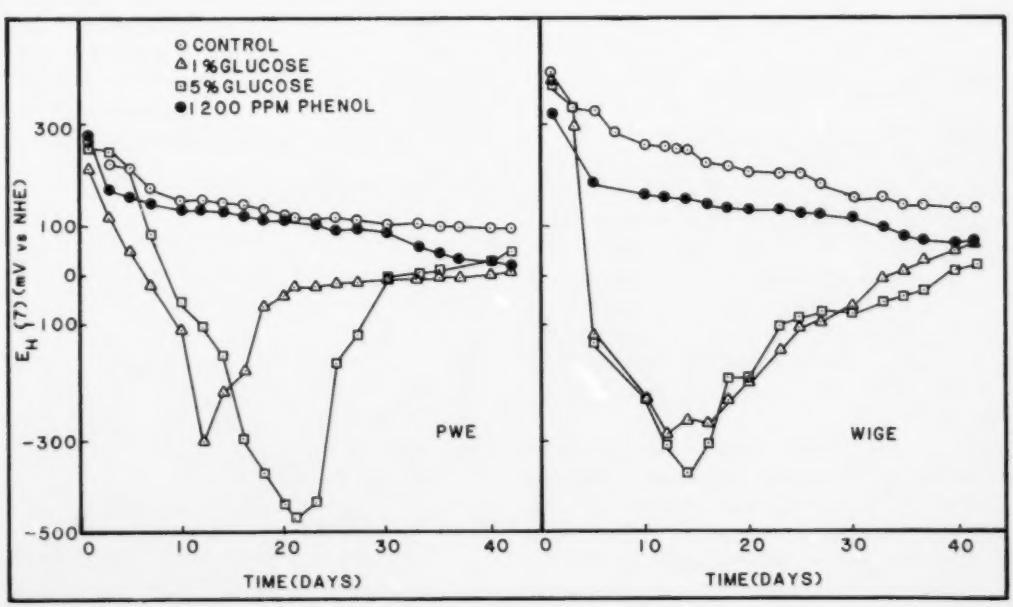

FIGURE 4. Variation of redox potential of soil samples during biodegradation. Soil samples were mixed well with organic compounds at 25% (W/W) moisture content and then flooded with water to obtain aneorobic condition.

tial. However, the observed change in E_H agreed qualitatively with that postulated elsewhere (1). For phenol samples, no such "dip" in E_H values was observed, although a degradation of as much as 60% occurred in about 30 days (Fig. 2). Since the degradation mechanism of phenol involves several intermediate steps prior to complete degradation (21), it is likely that the electrodes continued to show reducing environments until complete degradation was achieved. In other words, E_H measurements may indicate only the complete biodegradation of organic compounds into end products such as carbon dioxide and water. This hypothesis may be valid since, for phenol samples, E_H values never merged with those for the control, indicating a different redox condition in the system. This observation was confirmed by several experiments performed in our laboratory.

The data in Fig. 5 show the results of the E_H for the nonaerobic condition created by flooding from the top of the columns with solutions containing organic compounds. Although the same general trend was observed as in Fig. 4, the results were more difficult to interpret due to a nonuniform distribution of soil water in the columns. The presence of trapped air in the soil and the possible "channeling" of the solution may have caused different redox environments around the electrodes, resulting in "peaks" of E_H values. The reproducibility of experiments performed under this condition was inconsistent, obviously due to nonuniform distribution of water; therefore, this procedure is not recommended.

The data in Fig. 6 show the variation of ODR during biodegradation at a 25% moisture content. Phenol is a bacteriostat. Its degradation rate in soil is dependent on its concentration. Concentrations of phenol below 1 ppm in soil are "easily degraded" (22). Soil concentrations of phenol greater than 100 ppm are degraded slowly (19). The dramatic change for easily biodegradable glucose samples are observed for both types of electrodes. The initial decrease in ODR was due to rapid depletion of oxygen as a result of aerobic biodegradation. The completion of the degradation process was indicated when the curves (for glucose samples) merged with that of the control. As in the case of phenol, both PWE and WIGE show slow and incomplete degradation processes, resulting in intermediates which may still be consuming oxygen for further decomposition. The results of E_H and ODR measurements are, therefore, quite consistent and show a definite pattern for biodegradation processes for phenol and glucose.

The overall reproducibility of E_H and ODR measurements by various electrodes in the same sample was not good. The data presented in Figs. 4–6 are an average of results obtained with two electrodes of each type located in the same sample about six cm apart. The E_H values recorded showed a variation of $\pm 10\%$ or better for both PWE and WIGE, whereas the ODR varied by $\pm 17\%$ or better. Each electrode, however, showed the same pattern of change (in ODR and E_H) during the biodegradation processes. This variation was attributed more to the inhomogeneous nature of soil than to the response characteristics of the electrodes. In fact, more precise results are obtained by using a larger number of electrodes in the same sample. The performance of WIGE was

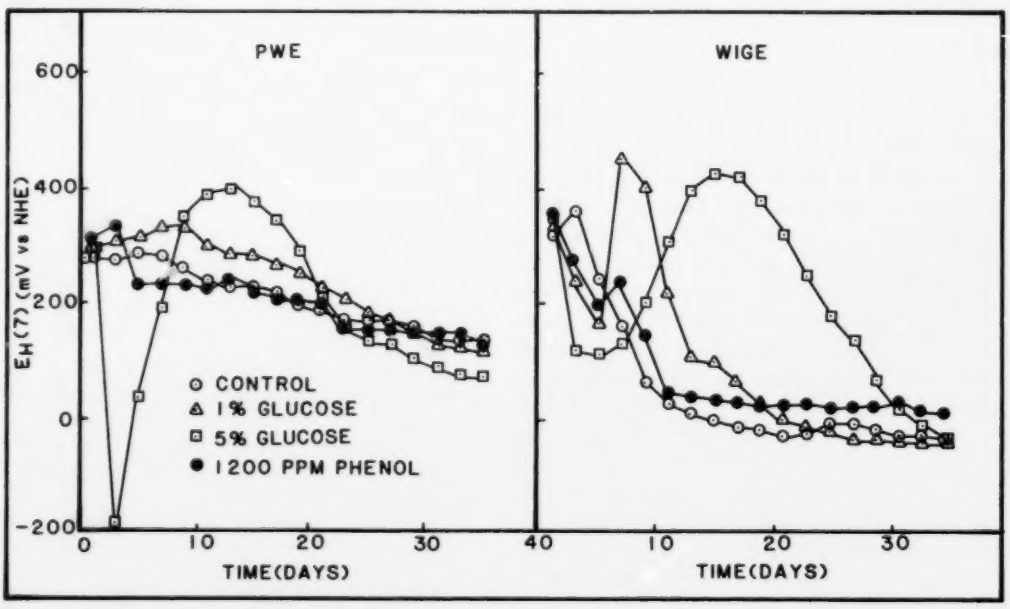

FIGURE 5. Variation of redox potential of soil samples during biodegradation. Solutions of organic compounds were added to the soil from the top to obtain anaerobic condition.

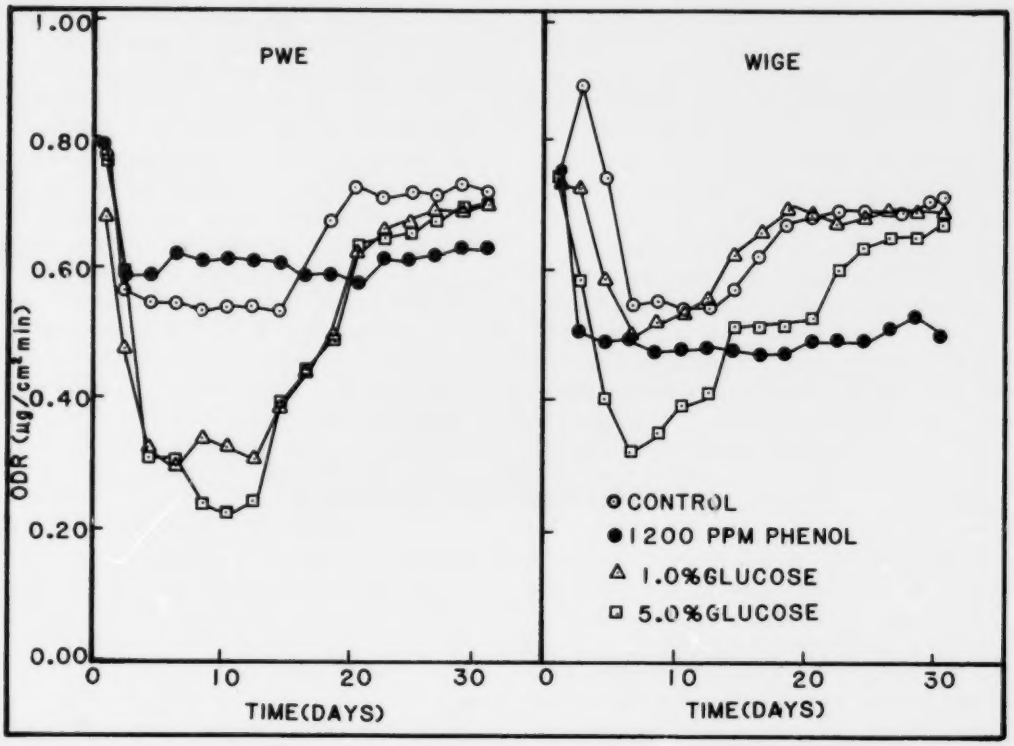

FIGURE 6. Variation of oxygen diffusion rate of soil samples amended with organic compound at 25% moisture content during biodegradation.

better than that of PWE, in terms of a more dramatic change in observed E_H and ODR; the reproducibility results, however, were not significantly better.

CONCLUSIONS

The results of these studies indicate that measurement of E_H and ODR can be used to monitor biodegradation of hazardous wastes in soil. Although this method appears to provide an indication for complete biodegradation, the course of the entire process can be monitored conveniently. The method is quite suitable for a landfarm situation where a larger number of probes can be distributed over the entire area, so that a better overall representation of the biodegradation process can be obtained through statistical evaluation. Moreover, development of electronics for remote-sensing of the electrodes with the use of a microcomputer for data collection and interpretation may provide a technique superior to the present procedure of periodic sample collection. Further studies are now being conducted on: (a) development of other graphite-based electrodes and their evaluation to monitor E_H and ODR with greater reproducibility and sensitivity, and (b) the variation of E_H and ODR as a function of organic and inorganic waste concentrations, loading rates of wastes, pH and the moisture content. A microcomputer-based instrument developed in our laboratory for automatic monitoring of E_H and ODR is being used for this purpose. Studies are also being conducted to observe the variation of both E_H and ODR for compounds which are known to biodegrade selectively under either aerobic or nonaerobic conditions (PCBs, for example).

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